Clin. exp. Immunol. (1972) **10,** 399–407.

ANTIGENIC RELATIONSHIP BETWEEN HUMAN IgE AND CANINE IgE

R. E. W. HALLIWELL, R. M. SCHWARTZMAN AND J. H. ROCKEY

Department of Veterinary Clinical Studies, University of Cambridge, Department of Clinical Studies, School of Veterinary Medicine, University of Pennsylvania, and Department of Ophthalmology, School of Medicine, Philadelphia, Pennsylvania

(*Received* 15 *July* 1971)

SUMMARY

Absorption of canine reaginic serum with anti-human IgE resulted in a substantial reduction in PK titre to ragweed. Anti-human IgE was also effective in eliciting reversed cutaneous anaphylaxis in the dog. This is conclusive evidence of antigenic similarity of canine and human IgE. The immunoelectrophoretic identification of canine IgE is described.

INTRODUCTION

The establishment of homology between antibody classes of different species forms an important aspect of comparative immunology. With the identification of IgE as the carrier of reaginic activity in man (Ishizaka, Ishizaka & Hornbrook, 1966; Stanworth *et al.*, 1967) the search for animal models of immediate type hypersensitivity mediated by a homologous antibody assumes great importance. The dog, which manifests a spontaneous atopic disease, many features of which are similar to atopic disease in man, lends itself particularly well to investigation in this respect (Schwartzman & Rockey, 1967; Schwartzman, Rockey & Halliwell, 1971; Halliwell & Schwartzman, 1971).

Vaerman (1970) has suggested three orders of criteria which should be applied when identifying homologous proteins in different species. First order criteria are immunological cross-reactivity and amino acid sequence homologies. Second order criteria include the specific association of a protein with a particular function, or the demonstration of unique additional determinants on a protein found in secretions (e.g. secretory IgA). These criteria have particular importance in the identification of IgA in different species. Third order criteria include a wide range of physicochemical and biological properties.

Stechschulte, Orange & Austen (1970) provided third order criteria to justify applying the term IgE to rat homocytotrophic antibody. First order criteria for establishing this homology have recently been provided by Kanyerezi, Jaton & Bloch (1971) who precipitated homocytotrophic antibody to *Nippostrongylus brasiliensis* with an anti-human IgE. Zvaifler &

Correspondence: Dr R. E. W. Halliwell, Department of Veterinary Clinical Studies, University of Cambridge, Madingley Road, Cambridge.

Robinson (1969) justify the use of the term rabbit IgE primarily because of physicochemical similarities with the human counterpart (third order criteria), but they also claimed that antigenic similarity exists (first order criteria). They based this on the reduction in homologous passive cutaneous anaphylaxis (PCA) titre from 1:20 to 1:10 that was obtained by absorption with anti-human IgE. Our experience with Prausntitz-Küstner (PK) tests in dogs suggests that such a minor reduction could be within the realm of experimental error, and unless consistently reproducible should not be regarded as significant.

Characterization of spontaneous anti-ragweed and induced anti-2,4-dinitrophenyl reaginic antibodies led us in a previous report to propose the term IgE for the canine reaginic antibody (Schwartzman *et al.*, 1971). The canine reaginic antibody has a sedimentation coefficient of 8–9S, is activated by heating to 56°C for 4 hr, and is mercaptoethanol sensitive (Schwartzman *et al.*, 1971; Rockey & Schwartzman, 1967). On Pevikon block zone electrophoresis it has γ_1 electrophoretic mobility (Schwartzman *et al.*, 1971; Halliwell, 1971). On diethylaminoethyl (DEAE) cellulose chromatography in phosphate buffer pH 8, the major part elutes between 10 and 35 millimoles (R. E. W. Halliwell, unpublished observation, 1971).

We now present first order criteria establishing this homology on the basis of immunological cross-reactions between human and canine IgE.

MATERIALS AND METHODS

Canine sera. Serum WT was obtained from a case of spontaneous atopic disease. The animal was multisensitive and gave strongly positive intradermal skin tests to the following extracts which contained 1000 protein nitrogen units (PNU) of allergen (Center Labs, Port Washington, N.Y.): dandelion, English plantain, goldenrod, human dander, kochia, lambs quarters, and ragweed. Serum D2702 was obtained from a dog suffering from severe roundworm infection (*Toxocara canis*), sarcoptic mange (*Sarcoptes scabei* var. *canis*), and lice infestation (*Trichodectes canis*).

Human sera. An IgE rich serum WX was kindly donated by Dr David Rowe, W.H.O. Immunoglobulin Research Centre, Lausanne, Switzerland. It was from a pool of African sera with an IgE content of approximately 20 μg/ml.

Agar diffusion studies. Immunoelectrophoresis was carried out by the microtechnique as described by Heremans (1960). Immunodiffusion studies were performed using 1·5% agar buffered with 30 mM barbital buffer, pH 8·6, layered on glass slides.

Fractionation of canine sera. The reagin rich fraction from serum WT (Fr.R) was obtained by filtration through three-in-series 4 × 60 cm columns of Sephadex G-200 as described previously (Schwartzman *et al.*, 1971). Those fractions prior to the commencement of the 7S peak which contained demonstrable PK reactivity to ragweed antigen were pooled. For the preparation of Fr.R from D2702 a three-step procedure was employed utilizing the established physicochemical properties of IgE. The protein that precipitated between 33·3% and 47·5% saturation with ammonium sulphate was equilibrated with 35 mM phosphate buffer, pH 8, and passed over a 4 × 40 cm column of DEAE-cellulose (Whatman, DE-52) equilibrated with the same buffer. The excluded protein was concentrated by negative pressure ultrafiltration and further fractionated on Sephadex G-200. The protein eluting immediately prior to the 7S peak was pooled as Fr.R.

Fractions of normal canine sera (NCS) for absorption of antisera. Canine IgG_{2ab} was obtained from a one-third saturated ammonium sulphate precipitate of NCS by exclusion from DEAE-cellulose at 5 mM phosphate buffer, pH 8. 7S-Fr.2 was the second half of the 7S peak of NCS following Sephadex G-200 gel filtration.

Pepsin digestion of canine IgG. Canine Fab_2 was prepared from IgG by digestion with 5% w/w pepsin (Koch-Light Laboratories Ltd, Colnbrook. England), in 200 mM acetate buffer, pH 4·5, for 36 hr at 37°C. Fab_2 was isolated from the reactant mixture by filtration through a 4 × 60 cm column of Sephadex G-100 in phosphate buffered saline (PBS), pH 7·2.

Antisera to human IgE. Antiserum WH was a sheep anti-human IgE, kindly donated by Dr David Rowe. This antiserum was raised against the IgE myeloma protein described by Johansson & Bennich (1967). Antiserum PH was a sheep anti-human IgE purchased from Pharmacia (Uppsala, Sweden). Antiserum NO was a swine anti-human IgE purchased from Nordic Pharmaceuticals and Diagnostics (Tilburg, The Netherlands).

C1703 and C2703 were antisera raised in chickens against precipitin bands resulting from agar immunodiffusion between sheep anti-IgE (WH) and human serum WX. Each chicken received two injections at monthly intervals of twenty carefully washed precipitin bands in complete Freund's adjuvant (CFA) (Difco, Detroit, Michigan). The resulting antisera were absorbed with normal sheep serum and canine IgG_{2ab}.

Antisera prepared against canine IgE. R9702 was raised in a rabbit against Fr.R from serum WT. The rabbit received three injections at 2-week intervals of approximately 1 mg of protein in 2 ml CFA administered at multiple subcutaneous sites. The antiserum was absorbed successively with pre-colostral newborn serum, 7S-Fr.2 and the fraction of NCS that eluted from DEAE-cellulose between 50 and 300 mM phosphate, pH 8.

R14701 was raised in a rabbit against Fr.R from D2702. The immunization schedule was as for R9702. The antiserum was absorbed with 7S-Fr.2.

Antisera R14701 and R9702 detected a protein of γ_1 electrophoretic mobility on immunoelectrophoresis against D2702 (Fig. 1). In anticipation that this could be IgE a chicken was immunized with this precipitin band. The chicken received sixteen of these bands intramuscularly in CFA followed 2 and 4 weeks later by a similar dose intravenously in PBS. The antiserum (C2714) was absorbed with normal rabbit serum and canine IgG_{2ab}.

Rabbit antisera prepared against other canine immunoglobulins and their fragments. R6711 was raised against a canine IgG_1 myeloma protein. The protein was isolated by gradient elution on DEAE cellulose and further purified by Pevikon block electrophoresis (Shandon). After absorption with Fab_2 it was monospecific for IgG_1.

R6701: A 33·3% ammonium sulphate precipitate of NCS was fractionated on DEAE cellulose using stepwise elution. The protein eluting between 100 and 200 mM phosphate buffer, pH 8, was further fractionated on Sephadex G-200, and the first half of the exclusion peak was used for immunization. The unabsorbed antiserum detected IgM, IgG_{2c} and IgG_{2ab}.

R13701: A 100 ml sample of canine milk was acidified with acetic acid. The precipitated casein was removed by centrifugation at 18,000 *g* for 30 min. The clarified whey was salted out with 40% ammonium sulphate. The precipitated protein was redissolved and fractionated on DEAE cellulose. The protein eluting between 50 and 90 mM phosphate buffer, pH 8, was further fractionated on Sephadex G-200. Antiserum R13701 was the product of immunizing a rabbit with the first peak. After absorption with 7S-Fr.2 it was specific for IgA.

R3711 was raised against IgG_{2ab} prepared as above. The antiserum was absorbed with Fab_2, and detected only IgG_{2ab}.

R2711 was raised against the protein from a 33·3% ammonium sulphate precipitate of NCS that eluted from DEAE-cellulose between 15 and 25 mM phosphate buffer, pH 8. After absorption with IgG_{2ab} the antiserum was specific for IgG_{2c}.

R4711 was raised against Fab_2.

The immunization schedules for all of these rabbits were as for R9702.

PK neutralization tests. Two-tenths ml of canine serum WT was mixed with an equal volume of each anti-canine IgE and anti-human IgE serum, and incubated at room temperature for 2 hr. Serum WT also was incubated with normal rabbit serum, normal sheep serum, normal chicken serum, and PBS as controls. After standing overnight at 4°C, any precipitate was removed by centrifugation at 3500 *g* for 15 min. Doubling dilutions then were made in PBS. Two large mixed breed non-atopic adult dog littermates were used as recipients. The hair of the lateral aspect of the chest was clipped and 0·2 ml of each dilution was injected intradermally. Twenty-four hours later the sites were challenged with 0·02 ml of ragweed extract (1000 PNU/ml, Centre Labs.). A positive reaction was recorded when the size of the weal at a sensitized site exceeded that of a control at an unsensitized site by 5 mm or more. Each serum/antiserum mixture and each control mixture was tested for residual PK reactivity in both dogs. Concordant results were achieved in most instances, but where they differed the titre recorded is the mean of the two tests.

Reversed cutaneous anaphylaxis. Ten-fold dilutions (in PBS) of each anti-human IgE and anti-canine IgE were injected intradermally into the lateral area of the chest of five normal dogs. Normal chicken, rabbit, pig and sheep sera also were included as controls for the appropriate antiserum. The highest dilution of each antiserum giving a weal within 15 min which exceeded the control by 5 mm or more was taken as the titration end point.

RESULTS

Characterization of antisera

(a) *Anti-human IgE.* All of the anti-human IgE sera detected the same protein in human serum WX (high in IgE), but failed to precipitate with normal human serum. Relative strengths in terms of antibody nitrogen content are not available, but a study of the position of the precipitin bands in relationship to the antigen and antibody wells suggested that antisera WH, NO and PH were of comparable strength, while antisera C1703 and C2703 were considerably weaker. No precipitate was visible with NCS or with canine sera WT or D2702.

(b) *Anti-canine IgE.* Antisera R9702 and R14701 detected two proteins in the majority of canine sera on immunodiffusion, and occasionally one weak band of γ_1 electrophoretic mobility on immunoelectrophoresis. In serum D2702, however, these antisera demonstrated a γ_1 protein which was clearly visible on immunoelectrophoresis (Fig. 1.). Antiserum C2714 was the product of immunizing a chicken with this band. After absorption with canine IgG it detected the same protein in D2702.

In immunodiffusion studies against a wide range of canine sera C2714 detected only one protein, and this protein was not demonstrable in all instances. This antiserum also neutralized PK activity (see below), and was thus adjudged monospecific for canine ε chains.

Antiserum R9702 precipitated with human serum WX, giving a band of identical specificity with that revealed by anti-human IgE serum WH. This, however, was the only anti-canine IgE to precipitate with human IgE.

PK neutralization. Of the anti-human IgE sera tested, only WH failed to produce an appreciable reduction in PK reactivity (Table 1). This effect was most marked with C1703, which reduced the PK titre from 1/5120 to 1/320.

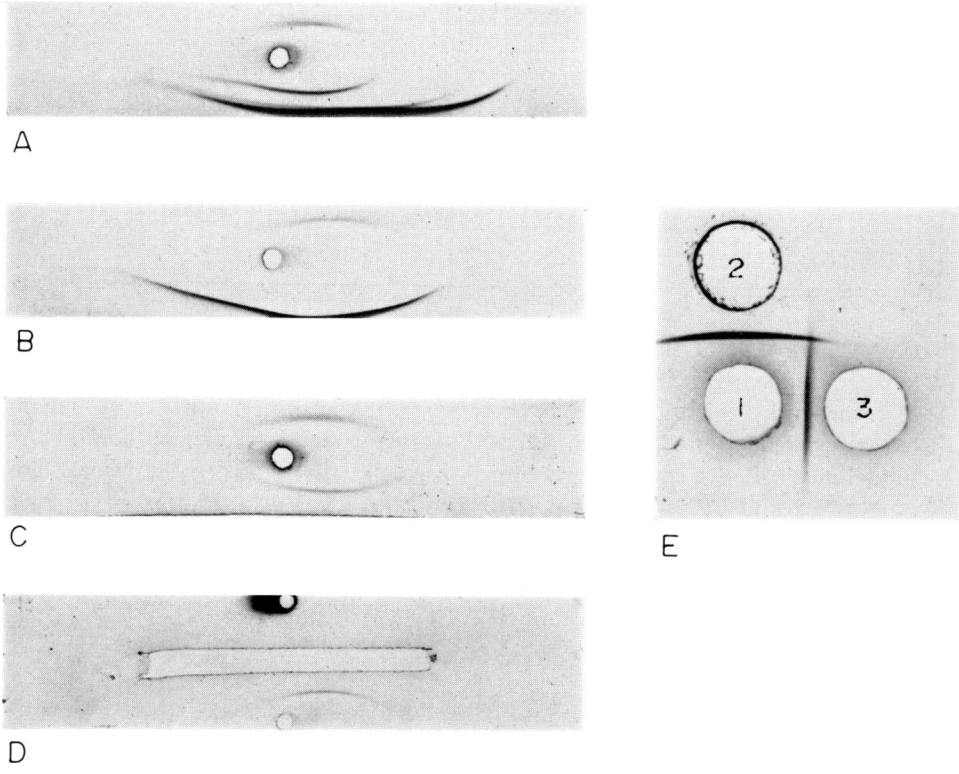

FIG. 1. Immunoelectrophoretic identification of canine IgE and its relationship to the other immunoglobulins of the dog. Anode to the left on all plates. A, B and C: Well contains serum D2702. Top troughs all contain R14701 (anti-IgE). Bottom troughs contain: A–R6702, which detects IgG_{2ab}, IgG_{2c} and IgM; B–R6711, which detects IgG_1; and C–R13702, which detects IgA. D: Top well contains D2702 heated to 56°C for 4 hr, bottom well contains unheated D2702. Trough contains R14701 (anti-IgE). E: Demonstrates the separate identity of IgE and IgA. Well 1 contains D2702, well 2 contains R14701 (anti-IgE) and well 3 contains R13702 (anti-IgA).

Not surprisingly anti-canine IgE had a more dramatic effect. Absorption with R9702 reduced the PK titre from 1/5120 to 1/40, and after absorption with R14701 and C2714, PK activity was undetectable at 1/10 dilution.

Reversed cutaneous anaphylaxis. The four anti-human IgE sera which reduced the titre of PK activity were tested for their ability to elicit reversed cutaneous anaphylaxis in five normal dogs (Fig. 2). All antisera elicited a weal and erythema response at a dilution of

TABLE 1. The effect of absorption with anti-human and anti-canine IgE on the ragweed PK titre of canine reaginic serum

PK titre on absorption	Anti-human IgE					Anti-canine IgE			
	WH	PH	NO	C2703	C1703	Controls	R9702	R14701	C2714
1/10240	−	−	−	−	−	−	−	−	−
1/5120	+	−	−	−	−	+	−	−	−
1/2560	+	−	−	−	−	+	−	−	−
1/1280	+	+	−	−	−	+	−	−	−
1/640	+	+	+	+	−	+	−	−	−
1/320	+	+	+	+	+	+	−	−	−
1/160	+	+	+	+	+	+	−	−	−
1/80	+	+	+	+	+	+	−	−	−
1/40	+	+	+	+	+	+	+	−	−
1/20	+	+	+	+	+	+	+	−	−
1/10	+	+	+	+	+	+	+	−	−

10^{-1} or greater in four of the five dogs. In dog 1, C1703 alone gave a positive result at 10^{-1}. In dog 3 a positive reaction was produced by high dilutions (10^{-2}–10^{-4}) of all four antisera. In general, the antisera which were most effective in neutralizing PK reactivity also were most effective in eliciting a reversed cutaneous anaphylactic reaction.

The anti-canine IgE sera elicited the response at greater dilutions than did the anti-human IgE sera. R14702 and C2714 gave a positive result in dog 3 at a dilution of 10^{-5}. It is noteworthy that all the antisera, both anti-human and anti-canine gave the lowest titres in dog 1 and the highest titres in dog 3.

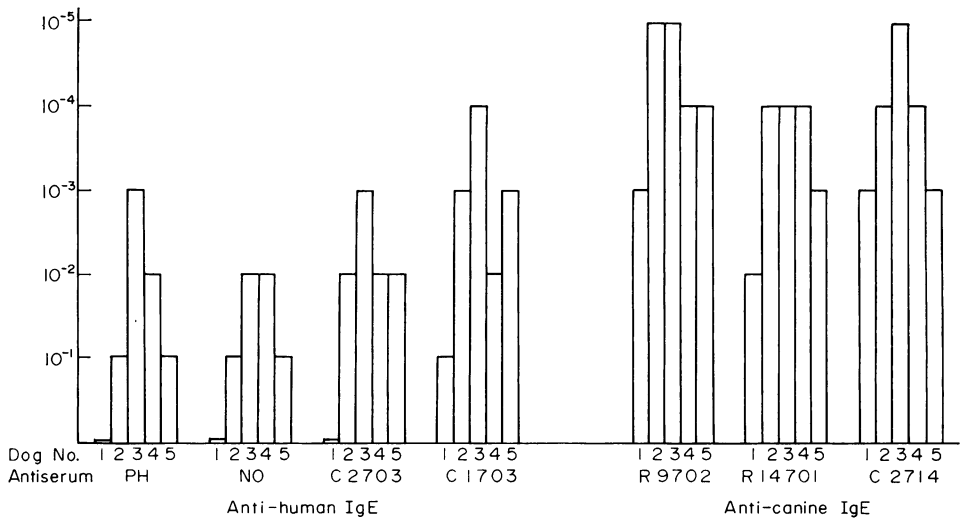

FIG. 2. The dilutions to which antisera to human and canine IgE were able to elicit reversed cutaneous anaphylaxis in five normal dogs.

Antisera to other canine immunoglobulins also were tested for their ability to elicit reversed cutaneous anaphylaxis. Heavy chain specific antisera to IgG_{2ab} (R3711), IgG_{2c} (R2711), IgG_1(R6711) and IgM (R6702 absorbed with 7S-Fr.2) all gave negative results at 10^{-1}. In two dogs, however, anti-IgA (R13702) elicited a response at 10^{-1}, and in one dog at 10^{-2}. The possibility that this could be due to contamination with anti-IgE was checked by absorbing the antiserum with Fr.R from D2702, which was high in IgE, but lacked demonstrable IgA. The same titre resulted. Anti-Fab_2(R4711) elicited a response in all animals at 10^{-1}, but the maximum dilution at which it was effective was 10^{-3} (dog 3).

Immunoelectrophoretic identification of canine IgE. The fact that chicken antiserum C2714, which resulted from immunizing a chicken with the γ_1 precipitin band revealed in canine serum D2702 by R14701 and R9702, neutralized PK activity provides convincing evidence that this band is canine IgE. Its relationship to the other immunoglobulin classes of the dog is shown in Fig. 1. The immunoelectrophoretic appearance is very similar to IgA, but Ouchterlony analysis confirms their non-identity (Fig. 1). The IgE band was not detectable after the serum was heated to 56°C for 4 hr (Fig. 1). Apart from a slight change in the electrophoretic mobility of IgM, the other immunoglobulin classes were unchanged by this treatment as judged by immunoelectrophoresis.

DISCUSSION

The importance of accepting rigid criteria for establishing homology of immunoglobulins in different species cannot be overemphasized. As recently as 1968 the canine homologue of IgA was incorrectly identified on inadequate evidence (Patterson, Roberts & Pruzansky, 1968; Johnson & Vaughan, 1967; Vaerman & Heremans, 1969). The same error was made in the horse where what is now known as IgT was originally proposed as the equine equivalent of IgA (Weir, Porter & Givol, 1966; Montgomery, Dorrington & Rockey, 1969; Rockey, Montgomery & Dorrington, 1970).

We have presented in a previous publication (Schwartzman *et al.*, 1971) evidence of third order criteria justifying our adoption of the term IgE for the canine reaginic antibody. This was based on a striking similarity of a wide range of physicochemical properties of human IgE and the presumed canine homologue. The substantial reduction in PK titre following absorption of canine reaginic serum with anti-human IgE demonstrates that canine IgE shares common antigenic determinants with human IgE. In this respect it is of interest that the chicken anti-human IgE sera, despite being the weakest as judged by immunodiffusion, were the most effective in neutralizing canine PK reactivity. Absorption of the antisera ensured that any cross reaction was due to common heavy chain antigenic determinants.

These findings constitute first order criteria in Vaerman's classification and are conclusive evidence of the homology of canine and human IgE.

The use of the reversed cutaneous anaphylaxis by anti-IgE was first described by Ishizaka & Ishizaka (1968). The reaction lends weight to the concept that target cells coated with IgE are present in the skin. That these target cells are mast cells is reinforced by the work of Hubscher, Watson & Goodfriend (1970) who showed that ragweed binding antibodies of the IgE class only could be demonstrated on the mast cells of monkey skin passively sensitized by human reaginic serum. Immunofluorescent studies on normal dog skin sensitized by serum from a dog with spontaneous atopic disease have shown that a

parallel situation exists in this species. Details of these findings will be reported at a future date. Of importance was the finding that dogs which gave positive reactions to intradermal injection of the higher dilutions of anti-canine IgE also tended to react to the higher dilutions of anti-human IgE. This correlation suggests that the reaction was immunologically specific, and is further evidence that the two proteins share common antigenic determinants.

It was of interest that anti-Fab_2 was relatively inefficient at inducing reversed cutaneous anaphylaxis. R4711 was a strong antiserum, precipitating at equivalence with 4 mg of IgG. The same observation was made by Ishizaka and Ishizaka (1968) who suggested that this was due to absorption of the antiserum by other immunoglobulins present in the skin. An alternative explanation could be that attachment at the Fab determinant site of the IgE molecule is less efficient at inducing mast cell degranulation than is attachment to the Fc portion of the molecule.

Intradermal injections of antisera specific for the other immunoglobulin classes failed to elicit a weal and erythema response at 1/10 dilution. The exception to this was anti-IgA which elicited a response in three animals, in one at 10^{-2}. Despite the fact that the amount of specific anti-IgA antibody nitrogen required to initiate reversed cutaneous anaphylaxis was probably some 1000 times greater than that of anti-IgE in the same dog, this result should not be ignored. Further work is required to establish whether it is an immunologically specific reaction or one simply due to generation of unusually large quantities of anaphylotoxin in absorbing the antiserum. Parish (1969), investigating a number of human reaginic sera, neutralized PK activity in baboons in two out of six sera from Ascarid hypersensitive patients by absorption with anti-IgA. The same antiserum was unable to absorb PK activity from a wide range of sera including some from grass allergic hay-fever sufferers. We have demonstrated previously that PK activity in canine atopic disease is unaffected by precipitation of serum IgA (Schwartzman *et al.*, 1971). However, in view of the prevalence of Ascarid infestation in the dog population, the possiblility of the existence of skin sensitizing anti-*Ascaris* antibodies of the IgA class should not be excluded.

The observations that IgE was no longer detectable on agar diffusion after heating to 56°C for 4 hr is in accordance with the finding in man. Ishizaka, Ishizaka & Menzel (1967) showed that antigen binding capacity under these circumstances was reduced but not lost. The implication of this is that the effect of heating is primarily to induce Fc configurational changes which affect both the antigenicity and the ability to fix to tissue.

As an aid to studies in comparative immunopathology the dog is of great interest in that it lives in an environment comparable if not identical with that of man, and is usually permitted a normal life span. A wide variety of spontaneous immunological disorders which include auto-allergic diesases (e.g. systemic lupus erythematosis), multiple myeloma, and atopic diseases, are thus allowed to express themselves. The antigenic challenge to which dogs are subjected is similar to that to which man is subjected. Thus of particular interest will be a study which is now proceeding of those conditions in the dog that lead to elevated levels of IgE.

ACKNOWLEDGMENTS

This investigation was supported by a grant from the Wellcome Trust, of which R.E.W.H. is a Research Fellow, and also by Research Grants GB-24701 from the National Science

Foundation, and AI-05305 from the National Institute of Allergy and Infectious Diseases, USPHS, and by a Public Health Service research career award (5-KO3-AM-16606) to R.M.S. from the National Institute of Arthritis and Metabolic Diseases, USPHS.

REFERENCES

HALLIWELL, R.E.W. (1971a) The immunology of allergic skin disease. *J. small Anim. Pract.* **12,** 431.

HALLIWELL, R.E.W. & SCHWARTZMAN, R.M. (1971) Atopic disease in the dog. *Vet. Rec.* **89,** 209.

HEREMANS, J. (1960) *Les Globulines Seriques du Systeme Gamma.* Editions Arscia S.A., Bruxelles.

HUBSCHER, T., WATSON, J.I. & GOODFRIEND, L. (1970) Target cells of human ragweed binding antibodies in monkey skin. *J. Immunol.* **104,** 1187.

ISHIZAKA, K. & ISHIZAKA, T. (1968) Reversed type allergic skin reactions by anti-γE-globulin antibodies in humans and monkeys. *J. Immunol.* **100,** 554.

ISHIZAKA, K., ISHIZAKA, T. & HORNBROOK, M. (1966) Physicochemical properties of human reaginic antibody. IV. Presence of a unique immunoglobulin as a carrier of reaginic activity. *J. Immunol.* **97,** 75.

ISHIZAKA, K., ISHIZAKA, T. & MENZEL, A. E. O. (1967) Physicochemical properties of reaginic antibody. VI. Effect of heat on γE, γG, and γA antibodies in the sera of ragweed sensitive patients. *J. Immunol.* **99,** 610.

JOHANSSON, S.G.O. & BENNICH, H. (1967) Immunological studies of an atypical (myeloma) immunoglobulin. *Immunology,* **13,** 381.

JOHNSON, J.S. & VAUGHAN, J.H. (1967) Canine immunoglobulins. I. Evidence for six immunoglobulin classes. *J. Immunol.* **98,** 923.

KANYENEZI, B., JATON, J.-C. & BLOCH, K.J. (1971) Human and rat γE: Serologic evidence of homology. *J. Immunol.* **106,** 1141.

MONTGOMERY, P.C., DORRINGTON, K.J. & ROCKEY, J.H. (1969) Equine antihapten antibody. The molecular weights of the subunits of equine immunoglobulins. *Biochemistry,* **8,** 1247.

PARISH, W.E. (1969) Detection of reagins. IgG, IgA, and IgM antibodies in human sera. *Int. Arch. Allergy,* **36,** 245.

PATTERSON, R., ROBERTS, M. & PRUZANSKY, J.J. (1968) Types of canine serum immunoglobulins. *J. Immunol.* **101,** 687.

ROCKEY, J.H., MONTGOMERY, P.C. & DORRINGTON, K.J. (1970) Equine antihapten antibody. Studies on the primary structure and conformation of the equine immunoglobulins. *Biochemistry,* **9,** 4310.

ROCKEY, J.H. & SCHWARTZMAN, R.M. (1967) Skin sensitising antibodies: A comparative study of canine and human PK and PCA antibodies and a canine myeloma protein. *J. Immunol.* **98,** 1143.

SCHWARTZMAN, R.M. & ROCKEY, J.H. (1967) Atopy in the dog. *Arch. Derm.* **96,** 418.

SCHWARTZMAN, R.M., ROCKEY, J.H. & HALLIWELL, R.E.W. (1971) Canine reaginic antibody—characterization of the spontaneous anti-ragweed and induced anti-dinitrophenyl reaginic antibodies of the atopic dog. *Clin. Exp. Immunol.* **9,** 549.

STANWORTH, D.R., HUMPHREY, J.H., BENNICH, H. & JOHANSSON, S.G.O. (1967) Specific inhibition of the Prausnitz-Küstner reaction by an atypical human myeloma protein. *Lancet,* **ii,** 330.

STECHSCHULTE, D.J., ORANGE, R.P. & AUSTEN, K.F. (1970) Immunochemical and biologic properties of rat IgE. *J. Immunol.* **105,** 1082.

VAERMAN, J.-P. (1970) Studies on IgA immunoglobulins of man and animals. Thesis, University of Louvain, Sintal-Louvain, Belgium.

VAERMAN, J.-P. & HEREMANS, J.F. (1969) The immunoglobulins of the dog. II. The immunoglobulins of canine secretion. *Immunochemistry,* **6,** 779.

WEIR, R.C., PORTER, R.R. & GIVOL, D. (1966) Comparison of the C-terminal amino acid sequence of two horse immunoglobulins IgG and IgG(T). *Nature (Lond.),* **212,** 205.

ZVAIFLER, N. J. & ROBINSON, J. O. (1969) Rabbit homocytotropic antibody—a unique rabbit immunoglobulin analogous to human IgE. *J. exp. Med.* **130,** 907.